Morphologic Identification of the OFF-Type Blue Cone Bipolar Cell in the Rabbit Retina

Pang-Chiung Liu[1] and Chuan-Chin Chiao[1,2]

Abstract

PURPOSE. Bipolar cells play major roles in transmitting visual signals from photoreceptors to ganglion cells and can be subdivided into at least 10 to 13 distinct types based on their morphology and physiology. This study aimed to morphologically identify the blue cone bipolar cells responsible for transmitting color signals in the rabbit retina.

METHODS. To find this cell type, bipolar cells were injected with a neuroanatomic tracer in the whole-mount rabbit retina and were subsequently labeled with peanut agglutinin and S-cone opsin to verify their cone selectivity.

RESULTS. Results indicated that all narrow- and medium-field bipolar cells showed no selectivity for cone type. Among widefield bipolar cells, one type made exclusive contact with Scones and was identified to be a blue cone bipolar cell. This cell type gave rise to four to five branchless primary dendrites that specifically contacted S-cone pedicles and their axons ramified in sublamina a of the inner plexiform layer.

CONCLUSIONS. In addition to the ON-type blue cone bipolar cell found in all mammalian retinas, the authors have identified the OFF-type blue cone bipolar cell in this study. Therefore, both ON and OFF blue cone bipolar cells are responsible for color information transmission in the rabbit retina. (*Invest Ophthalmol Vis Sci.* 2007;48:3388–3395) DOI:10.1167/iovs.06-1531

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The mammalian retina contains a huge diversity of cell types and functionally distinct synaptic pathways.[1] A typical rabbit retina contains only one type of rod and two types of cone photoreceptor cells, but there are as many as 13 different types of bipolar cells, including 12 types of cone bipolar cells and one type of rod bipolar cell.[2–4] This diversity of bipolar cells in the retina has been suggested for the initiation of parallel processing early in the visual system.[1,5]

Most mammals have two types of cone photoreceptors, one spectrally tuned to medium or long wavelengths (i.e., M- or L-cones) and the other tuned to short wavelengths (i.e., S-cones or blue cones). In trichromatic primates, however, three types of cone photoreceptors (L-, M-, and S-cones) are present in the retina.[6,7] In primates and nonprimates, S-cones typically make up less than 10% of all cone populations.[8] Thus, identifying the bipolar cell type whose dendrites exclusively connect to this sparsely distributed S-cone mosaic (responsible for the blueyellow opponency pathway) has been difficult.[9] In primates, a distinctive ON-type of blue cone bipolar cells was first identified by Golgi staining[10] and subsequently was confirmed with an antiserum that recognizes glycine-extended cholecystokinin (CCK) precursors to show its exclusive S-cone contacts.[11,12] In a recent study, Haverkamp et al.[13] used transgenic mice expressing Clomeleon (a chloride-sensitive fluorescent protein) to selectively label blue cone bipolar cells and showed that this type of bipolar cells contacts S-cones exclusively across all retinal areas. In ground squirrels, Li and DeVries[14] reported that one type of ON cone bipolar cells receives inputs exclusively from S-cones by recording from cone-bipolar cell pairs. Mouse and ground squirrel studies indicate that these bipolar cells with exclusive S-cone connectivity are morphologically similar to the blue cone bipolar cells identified in primates, thus representing the evolutionarily conserved color system of the mammalian retina.

Rabbits, like other nonprimate mammals, have only two types of cone photoreceptors in the retina. Behavioral evidence[15,16] and ERG experiments[17] indicate that rabbits have dichromatic color vision. Despite the fact that most cell types in the rabbit retina have been carefully characterized,[18] there are only a few reports about the neural circuitry of color processing in rabbits. In physiological studies, the potential color-encoding ganglion cells in the rabbit retina have been found by Caldwell and Daw,[19] De Monasterio,[20] and Vaney et al.[21]; however their morphologic identities were not clear. In morphologic studies, Famiglietti4 reported two types of widefield bipolar cells as the color-encoding bipolar cells (both ONand OFF-types) based on certain morphologic features of Golgi staining, but their S-cone contacts were not examined. Jeon and Masland[22] used biocytin to selectively label an ON-type of wide-field bipolar cell in the rabbit retina and postulated that this cell type represented the population of blue cone bipolar cells. Recent results of MacNeil and Gaul (MacNeil MA et al. *IOVS* 2006;47:ARVO E-Abstract 147) confirmed that these biocytin labeled wide-field bipolar cells indeed contacted S-cones exclusively. However, physiological evidence was lacking, and S-cone inputs to all bipolar cell types have not been systematically examined. Furthermore, morphologic characteristics of the proposed OFF-type blue cone bipolar cells4 were inconsistent with other mammalian results.[13,14,23,24] This uncertainty warrants a thorough investigation to reexamine the color-encoding bipolar cells in the rabbit retina.

Our goal in this study was to morphologically identify the cone contacts of bipolar cells in the rabbit retina to see which type or types are involved in color processing. We injected bipolar cells with neuroanatomic tracer (Neurobiotin; Vector Laboratories, Burlingame, CA) in the whole mount-retina preparation and immunocytochemically labeled S-cones to examine synaptic connections between the injected bipolar cells and S-cone photoreceptors.[25]

Although nearly all bipolar cells were nonselective for cone type, we found an OFF-type of wide-field bipolar cells that gave rise to four to five branchless primary dendrites to specifically contact S-cone pedicles. Our results provide direct support that the OFF-type wide-field blue cone bipolar cell is responsible for the cone-selective retinal circuitry in rabbits. A preliminary account of this finding has been given in abstract form (Chiao CC et al. *IOVS* 2006;47:ARVO E-Abstract 150).

Methods

Retina Preparation

All retinal neurons in both eyes of adult New Zealand White rabbits (weight range, 0.8 – 1.2 kg) were labeled by intraocular injection of 4 μg 4,6-diamidino-2-phenylinodole (DAPI; Sigma, St. Louis, MO) under general anesthesia (intramuscular injection of 70–75 mg/kg ketamine and 15 mg/kg xylazine) 1 to 3 days before experiments. On experimental day, the animals were deeply anesthetized by intramuscular injection of ketamine (150 mg/kg) and xylazine (30 mg/kg). Eyes were anesthetized with topical application of 0.5% proparacaine hydrochloride ophthalmic solution (Alcaine; Alcon-Couvreur, Puurs, Belgium). After enucleation and hemisection of each eye, the vitreous was removed and the retina was carefully isolated from the retinal pigment epithelium. The free-floating retinas were immersed in oxygenated (95% O2 and 5% CO2) modified Ames medium (120 mM NaCl, 3.1 mM KCl, 0.5 mM KH_2PO_4, 1.2 mM $MgSO_4$, 1.15 mM $CaCl_2$, 6.0 mM D-glucose, 23 mM $NaHCO_3$, pH 7.7). Each animal was humanely killed with an overdose of ketamine. All procedures were approved by the institutional animal care and use committee and were conducted in accordance with the ARVO Statement for Use of Animals in Ophthalmic and Vision Research.

Tracer Injection of Bipolar Cells

A small piece of retina (approximately 5 × 5 mm) from the dorsal side was cut and attached to a coverslip, photoreceptor layer down, with tissue adhesive (Cell-Tak; BD Biosciences, San Jose, CA). The dorsal side retina was chosen because S-cones are sparsely distributed in this area and no coexpression of M- and S-cone opsin in single photoreceptors is found there.[25–29] This preparation was transferred to a custom-made chamber mounted on the stage of a fluorescence microscope (Axioskop 2 FS Plus; Carl Zeiss, Oberkochen, Germany) and was superfused at 2.5 to 3.5 mL/min with oxygenated modified Ames medium at room temperature.

DAPI-labeled retinal neurons were viewed through a 40× water immersion objective (Achroplan, NA 0.8; Carl Zeiss) and a filter set appropriate for DAPI and lucifer yellow. Micropipettes were pulled from the thin-wall aluminosilicate glass capillaries with filament (outer diameter, 1.0 mm; inner diameter, 0.68 mm; Sutter Instrument, Novato, CA) using a programable puller (Flaming-Brown P97; Sutter Instrument). They were back-filled with 2% lucifer yellow (Sigma) and 4% tracer (Neurobiotin; Vector Laboratories) in 0.1 M Tris buffer for microinjection. An intracellular amplifier (Neuroprobe Amplifier 1600; A-M Systems, Carlsborg, WA) was used to perform the iontophoresis. Cell somata located at the outer two thirds of the inner nuclear layer (INL; near the sclera side) were selected by the DAPI-labeling features. A biphasic current (1–3 nA at 10 Hz) was used to inject tracer

(Neurobiotin; Vector Laboratories) and lucifer yellow for 5 to 30 seconds. To ensure a good quality of dye injection, the morphology of the dendritic and axon terminals were checked frequently during injection, and the injecting current was turned off when the terminal dendrites were completely filled. When several bipolar cells in a piece of tissue were filled, the retina was rinsed in 0.1 M phosphate buffer for further processing.

Although wide-field bipolar cells in the rabbit retina were rarely encountered,[2–4] we used several DAPI-labeling features of neurons in the INL[30] to facilitate identification of the blue cone bipolar cells. These included soma size, shape, and brightness. For example, we found that soma sizes of wide-field bipolar cells tended to be similar to, or slightly smaller than, those of A-type horizontal cells, but soma shape was more circular than in A-type horizontal cells. Soma brightness of wide-field bipolar cells under the DAPI filter was medium compared with the brightness of other somata at the same level of the INL. Relying on these DAPI-labeling features, we successfully injected 17 wide-field bipolar cells.

PNA Labeling of Cone Photoreceptors

Retinal pieces with well-injected bipolar cells were incubated in rho-damine-tagged peanut agglutinin (PNA; Vector Laboratories) for 75 minutes at room temperature to selectively label all cone photoreceptors. [31] The incubating solution (1–2 mL) was made up of 200 μg PNA and 1 mg bovine serum albumin per 1 mL of 0.1 M phosphate buffer (PB). After incubation, retina pieces were washed thoroughly in 0.1 M PB, postfixed for 50 minutes in 4% paraformaldehyde in 0.1 M PB, and further washed in 0.1 M PB.

Immunocytochemical Labeling of S-Cones and M-Cones

To identify the blue cones (S-cones) among the PNA-labeled cones, we used goat polyclonal antiserum against rat, mouse, and human S-cone opsin (sc-14363; Santa Cruz Biotechnology, Santa Cruz, CA). This antibody has been reported to label S-cones specifically and reliably in some mammalian species[13] (Yang HJ et al. *IOVS* 2004;45:ARVO E-Abstract 5310). Nonspecific binding sites were blocked by incubation with 4% normal donkey serum (Jackson ImmunoResearch Laboratory, West Grove, PA), 0.1% Triton X-100, and 0.05% sodium azide in 0.1 M PB for 2 hours. After blocking, retinal pieces were incubated 2 to 3 days in primary antiserum (diluted 1:200 in the blocking solution); this was followed by further thorough washing in 0.1 M PB. Retinal pieces were subsequently incubated overnight in FITC-conjugated donkey anti– goat IgG (diluted 1:100 in the blocking solution; Jackson ImmunoResearch Laboratory). After further washing in 0.1 M PB, tracer (Neurobiotin; Vector Laboratories)–filled bipolar cells were visualized by incubation overnight with FITC-conjugated streptavidin (diluted 1:50 in 0.1 M PB with 0.1% Triton X-100; Vector Laboratories). Finally, retinal pieces were flat mounted, vitreal side up, on modified glass slides (lines of nail polish served as spacers to preserve the vertical structure of the outer retina) and were coverslipped (Vectashield; Vector Laboratories) for confocal imaging.

In a separate experiment, we examined the topographic distribution of S-cones and M-cones across the entire rabbit retina by double labeling S-cones with the same goat anti–S-opsin (1:200) described and double labeling M-cones with the rabbit polyclonal antiserum against the human M/L-cone opsin (AB5405; 1:200; Chemicon International Inc., Temecula,

CA). Similar immunocytochemical labeling steps were applied, except that the mounting was performed with the vitreal side down to facilitate visualization of photoreceptor outer segments.

Confocal Microscopy and Image Acquisition

All images were acquired with a confocal microscope (LSM 5 Pascal; Carl Zeiss) with a 63× objective (C-Apochromat, NA 1.2; Carl Zeiss). A series of z-stack images (1-μm interval) was taken from the focal plane of axon terminals of the bipolar cell, through dendrites of the bipolar cell and cone pedicles of photoreceptors, all the way to cone outer segments. Therefore, it was possible to show the cone types contacted by the dendritic terminals of bipolar cells, despite the fact that the cone outer segments usually were laterally displaced from the cone pedicles. To determine the stratification of bipolar cells in the inner plexiform layer (IPL), the ganglion cell layer (GCL) uppermost boundary was set as 100% and the INL lowest boundary was set as 0% using DAPI staining. The axon terminal depth of the bipolar cell was then measured and calculated as a percentage in the IPL.

Evaluation and Analysis of Bipolar Cell Types

To determine cone contacts of injected cells accurately, only bipolar cells whose processes were completely filled and whose pedicles and outer segments of cone photoreceptors were clearly labeled were used in this study. These were judged by the presence of claw structures of dendritic terminals and aggregates of axon terminals. Cells not meeting these criteria were excluded from analysis. We classified a bipolar cell as a genuine blue cone bipolar cell only if all its dendrites contacted S-cones exclusively in the dorsal side of the retina. All other bipolar cells were classified into groups according to cell types described in MacNeil et al.[2] To improve image quality, intensity and contrast were adjusted (Adobe PhotoShop CS version 8.0.1; Adobe Systems, Mountain View, CA). For calculating the sizes and diameters of dendrites and axon terminals of bipolar cells, we connected all terminals together to form polygons and used them to estimate these two parameters (LSM 5 image viewer, version 3.1.0.99; Carl Zeiss).

Results

Topographic Distribution of S-Cones and M-Cones across the Retina

It has been shown that S-cones are distributed unevenly across the rabbit retina. The dorsal retina is sparsely populated with S-cones, and the ventral-most retina is rich in them.[25–29] This uneven cone distribution pattern is not uncommon in mammalian retinas[32] but is sensitive to the antibody used. Because we used a relatively new commercially available antibody to label S-cones[13] (Yang HJ et al. *IOVS* 2004;45:ARVO E-Abstract 5310), it was worthwhile to reexamine this unique S-cone distribution across the rabbit retina.

With the use of a goat polyclonal antiserum against S-opsin (Santa Cruz Biotechnology, Santa Cruz, CA) and the rabbit polyclonal antiserum against M-ospin (Chemicon, Temecula, CA), we double labeled the retina for S-cones and M-cones in four regions (dorsal, visual streak, ventral, and blue streak). Five sites in each region (145 ×145 μm^2) were imaged, and

the densities of S-cones and M-cones were computed (Table 1; Fig. 1). Consistent with previous studies, the rabbit retina exhibited a dominance of M-cones over S-cones in the dorsal side (5:1), in the visual streak (11:1), and in the ventral side (3:1).[25–29] In the blue streak, blue cones were highest in density (11,000/mm^2), and there was little dominated M-cone opsin expression (Fig. 1). It should be noted that in our study and others,[25,29] almost all cones showed weak immunoreactivity to M-cone opsin in the blue streak, but only a subset of cones expressed M-cone opsin strongly in this area (Fig. 1, lower-right panel). For the dual opsin– expressing cone population, there was a gradient from zero in the dorsal side to a high ratio (approximately 18% or 97% of total cone population, depending on whether counts included cells with weakly expressed M-cone opsin) in the blue streak (Table 1). It has been reported that the morphologies of blue cone bipolar cells are similar in different locations of the retina[12,13]; therefore, we chose to inject cells in the dorsal retina. This is because the absence of coexpression of S- and M-cone opsin in individual photoreceptors and the sparsely distributed S-cones in the dorsal side mark this part of the rabbit retina an unambiguous area to seek blue cone bipolar cells in the present study.

TABLE 1. S- and M-Cone Distribution in the Rabbit Retina

Cell Location	S-Cones (cell/mm^2)	M-Cones (cell/mm^2)	Duel-Expressing Cones (cell/mm^2)	Total Cones (cell/mm^2)	Duel-Expressing Cone Ratio (%)
Dorsal side	1,027 ± 230	5,470 ± 571	0 ± 0	6,497 ± 512	0.00
Visual streak	885 ± 278	9,969 ± 1,243	48 ± 60	10,806 ± 1,429	0.44
Ventral side	2,197 ± 663	7,391 ± 1,003	837 ± 316	8,751 ± 1,356	9.56
		2,293 ± 418*	1,979 ± 353*		17.56*
Blue streak	10,958 ± 1,328	11,272 ± 1,395†	10,958 ± 1,328†	11,272 ± 1,395	97.21†

* Only M-cones with highly expressed M-cone opsin.
† All M-cones with weakly and highly expressed M-cone opsin.

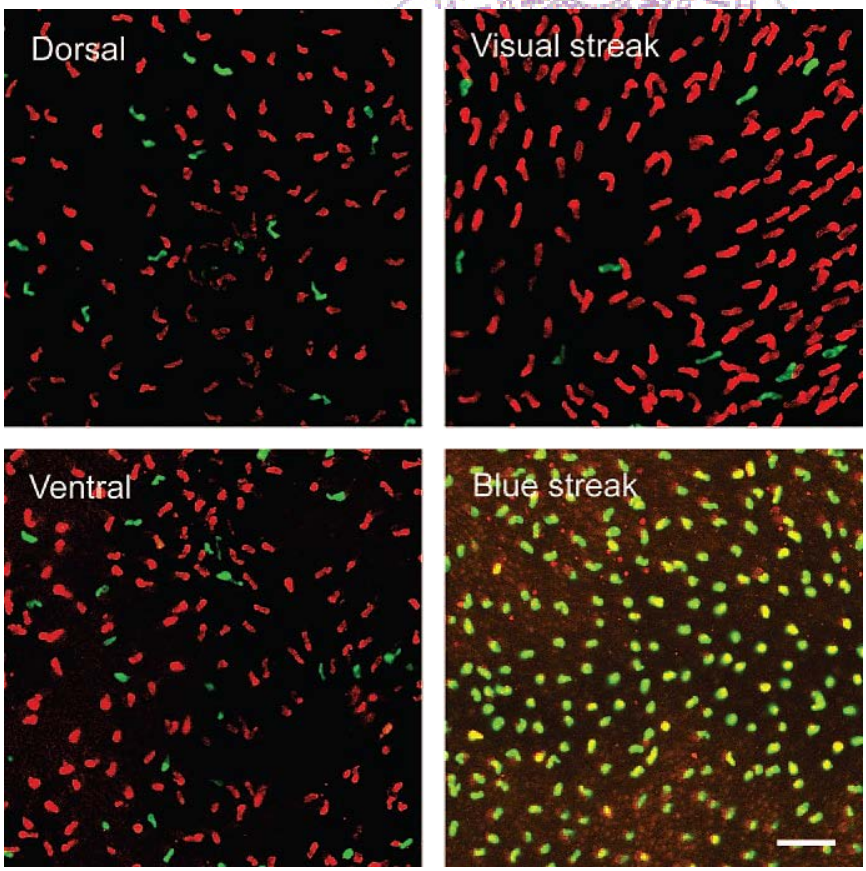

FIGURE 1. Topographic distributions of S-cones and M-cones vary across the retina. Four represented regions of the retina (dorsal, visual streak, ventral, and blue streak) were double labeled by specific antibodies against S- and M-cones. *Green*: S-cones labeled by the anti–S-opsin. *Red*: M-cones labeled by the anti–M-opsin. *Yellow*: coexpression of S- and M-opsin. Scale bar, 20 μm.

Nonselective Cone Connection in Narrow- and Medium-Field Bipolar Cells

We injected 87 bipolar cells that were filled well enough to be classified according to the types described by MacNeil et al.[2] Except for one type (the wide-field BP), we identified 11 cone bipolar cell types and one rod bipolar cell type (at least two cells in each type; cell types and numbers are listed in Table 2). Among these 12 bipolar cell types, all had narrow or medium fields of axon terminals (i.e., diameter is smaller than 50 μm). Among these 11 cone bipolar cell types, all received inputs from all cones in their dendritic fields and were not selective for S-cones or M-cones, which is consistent with previous studies.[4,23] Thus, they are not candidates for the blue cone bipolar cells. For example, Figure 2 is a collapsed confocal image of a $CBb3_n$ bipolar cell, in which all cone pedicles and outer segments of blue cones were simultaneously labeled. This $CBb3_n$ bipolar cell contacted all nine cone pedicles in its dendritic field, but none were blue cones.

S-Cone–Selective Contacts in Some Wide-Field Bipolar Cell Types

Although wide-field bipolar cells were rarely encountered in previous studies,[2–4] we have successfully injected 17 cells whose axonal field diameters were larger than 50 μm and thus qualified as wide-field bipolar cells. We classified these 17 identified wide-field bipolar cells into 4 groups (Table 3), based on their S-cone contacts and axon terminal stratifications.

Two wide-field bipolar cells observed in this study showed specific S-cone contacts (Fig. 3). We termed them the blue cone bipolar cells (or BB cells). These two BB cells had large axon terminals (60 and 80 μm in axonal field diameter) and dendritic fields (40 and 50 μm in diameter). They had only four to five unbranched primary dendrites that varied in length and contacted S-cone pedicles exclusively at their terminal claw structures. The axon terminals of the BB cells were stratified in sublamina 1 of the inner plexiform layer (IPL) and ended with some varicosities. Thus, they are considered to be an OFF cone bipolar cell type.

Besides these two BB cells, we also found five wide-field bipolar cells whose dendritic morphologies and axon terminal stratifications were similar to the BB cells described except that their primary dendrites contacted only one to two available S cones in their dendritic fields (Fig. 4). We termed them BB-like bipolar cells. These BB-like cells probably belong to the same group of blue cone bipolar cells. We address this possibility in the Discussion.

TABLE 2. Summary of Narrow- and Medium-Field Bipolar Cells Examined

Cell Type	No. Cells Injected	Cone Connectivity
CBa1	9	S, M
$CBa1_w$	8	S, M
CBa1–2	9	S, M
$CBa1–2_n$	5	S, M
CBa2	8	S, M
$CBa2_n$	7	S, M
$CBb3_n$	8	S, M
CBb3	10	S, M
CBb3–4	7	S, M
CBb4	8	S, M
CBb5	6	S, M
Rod bipolar	2	None

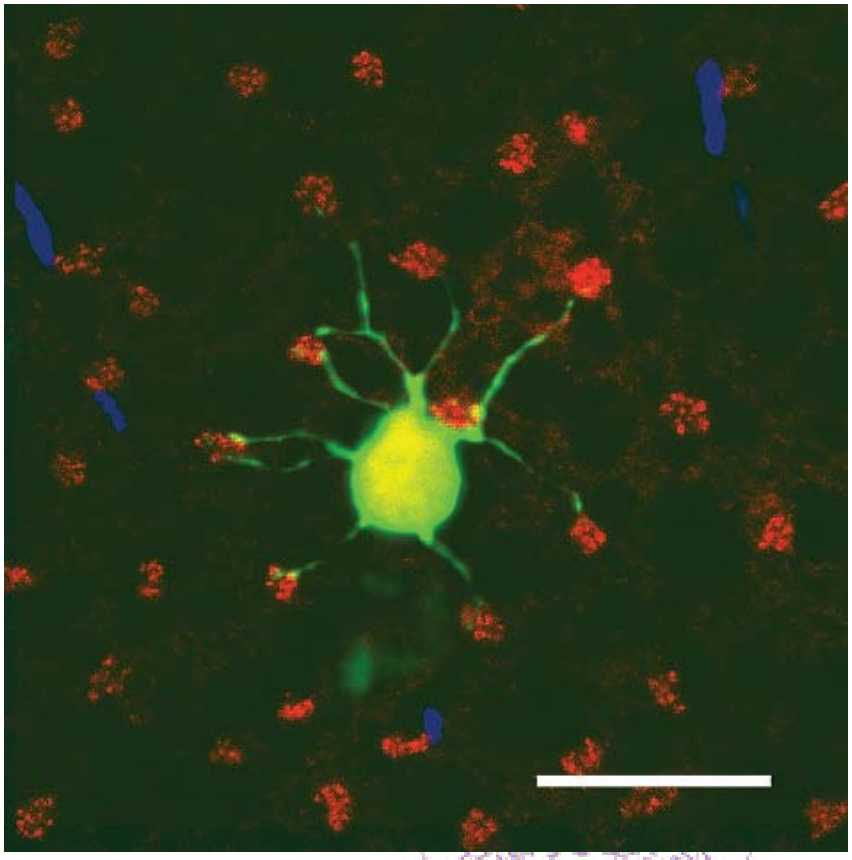

FIGURE 2. A narrow-field bipolar cell ($CBb3_n$) makes synaptic connections to all cones in its dendritic field. This image was collapsed from a confocal z-stack (1-µm interval) of the INL and OPL to show the tracer-injected bipolar cell (*green*), the PNA-labeled cone pedicles (*red*), and the anti–S-opsin–labeled outer segments of S cones (*blue*). Given that outer segments were not exactly on top of cone pedicles, the image of outer segments was shifted slightly to match the locations of underlying cone pedicles. In this image, the $CBb3_n$ bipolar cell contacted all nine cone pedicles in its dendritic field but no S-cone pedicle. Scale bar, 20 µm.

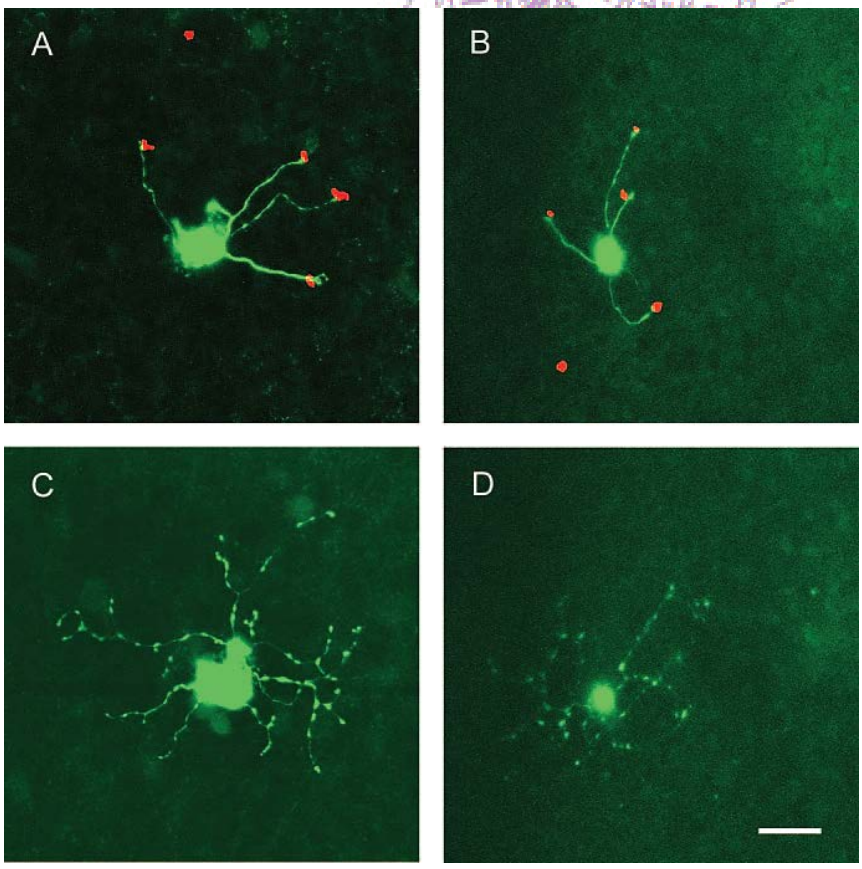

FIGURE 3. Morphology of two identified blue cone bipolar cells (the BB cells). (**A**, **B**) Collapsed confocal stacks of the INL and OPL superimposed with the outer segments of S-cones (*red*) to show dendrites of two identified blue cone bipolar cells and their selective cone contacts. (**C**, **D**) Collapsed confocal stacks of three optical sections (1-_m interval) to show axon terminals of two identified blue cone bipolar cells. Scale bar, 20µm.

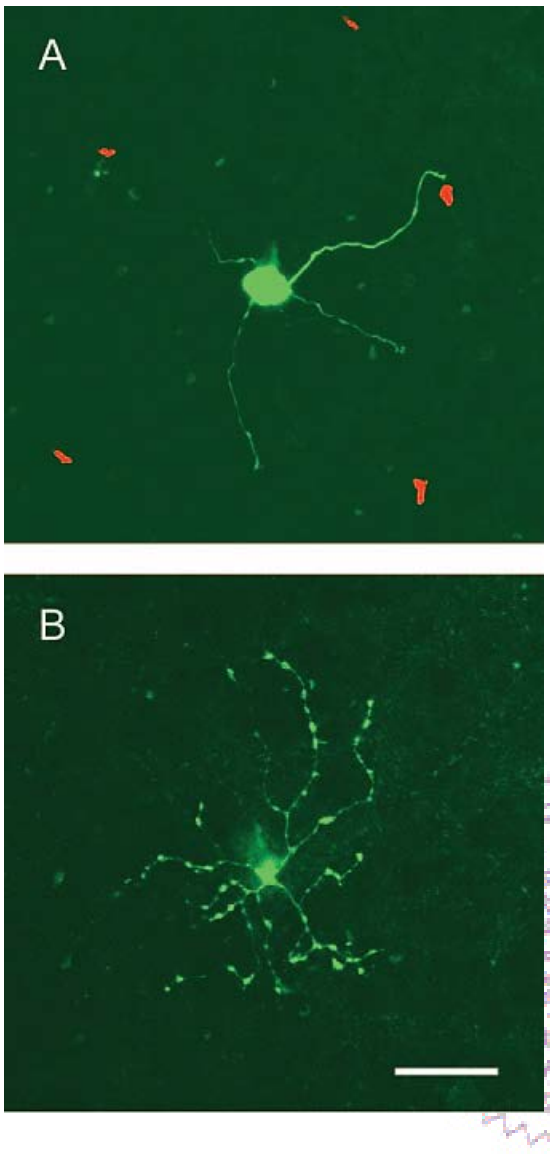

FIGURE 4. Morphology of the identified BB-like bipolar cell. (**A**) Collapsed confocal stacks of the INL and OPL superimposed with the outer segments of S-cones (*red*) to show dendrites of the identified BB-like bipolar cell and its partial S-cone contacts. (**B**) Collapsed confocal stacks of three optical sections (1-μm interval) to show axon terminals of the identified BB-like bipolar cell. Scale bar, 20 μm.

We also identified six wide-field bipolar cells whose axon terminals stratified in sublamina a of the IPL, and four widefield bipolar cells whose axon terminals stratified in sublamina b of the IPL (Fig. 5). These 10 wide-field bipolar cells had no selective cone contacts; thus, they are not candidates of the blue cone bipolar cells in this study. We termed them the WFa cells and the WFb cells to distinguish them from the wa cells and the wb cells identified in Famiglietti.[4] The WFa and WFb cells are similar in their dendritic morphologies and differ only in their axon terminal stratifications. They had many branched dendrites and received all cone inputs in their dendritic fields. The axons of the WFa and WFb cells had fewer varicosities on their terminals but more loops in processes.

However, we did not find any wide-field bipolar cell whose morphology resembles the wb cells in Famiglietti,[4] the biocytin labeled wide-field bipolar cells in Jeon and Masland,[22] or the wide-field BP cells in MacNeil et al.,[2] though they certainly exist in the dorsal retina (MacNeil MA et al. *IOVS* 2006;47: ARVO E-Abstract 147).

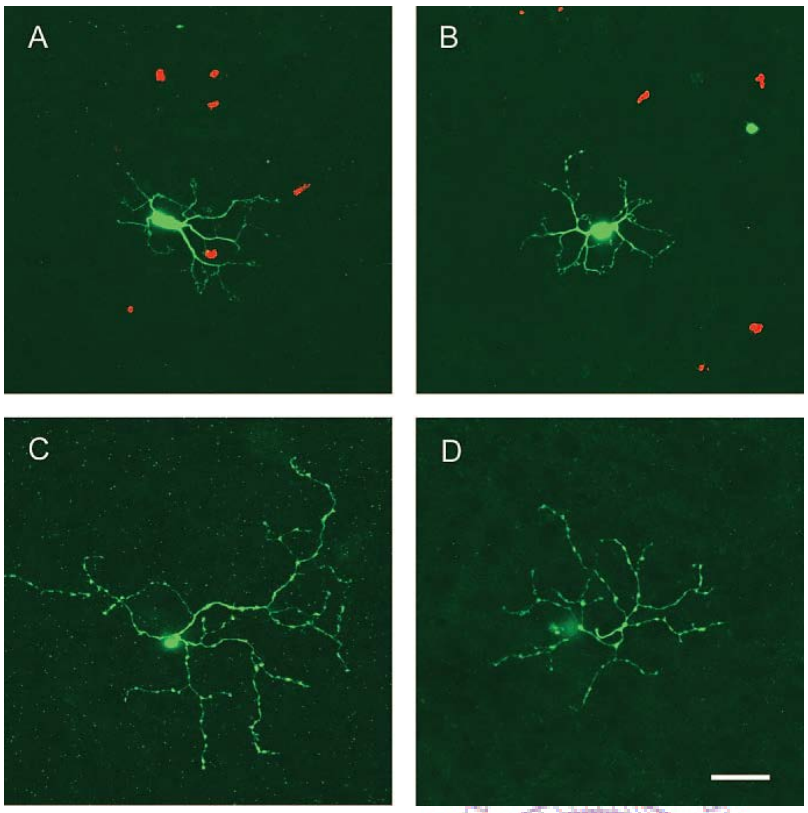

FIGURE 5. Morphology of the identified WFa and WFb bipolar cells. (**A**, **B**) Collapsed confocal stacks of the INL and OPL superimposed with the outer segments of S-cones (*red*) to show dendrites of the WFa and WFb bipolar cells and their nonselective cone contacts, respectively. (**C**, **D**) Collapsed confocal stacks of three optical sections (1-μm interval) to show axon terminals of the WFa and WFb bipolar cells, respectively. Scale bar, 20 μm.

Discussion

Color information processing starts at the first synapses between photoreceptors and cone bipolar cells in the mammalian retina. In primates and nonprimates, the bipolar cell responsible for color circuitry has been shown consistently as the ON-type blue cone bipolar cell.[13,14,23] By injecting bipolar cells and immunostaining S-cones in the dorsal side of the whole-mount rabbit retina, we have identified an OFF-type of wide-field bipolar cells whose dendritic terminals contact Scone pedicles exclusively. This bipolar cell type is likely to provide short-wavelength signals to the underlying color-encoding ganglion cells in the rabbit retina.

Similarity between the BB Cells and the BB-like Cells

Although we separated BB cells and BB-like cells based on complete S-cone contacts (Table 3), the similar dendritic morphologies and axon terminal stratification patterns encouraged us to group them into the same cell type. Supporting evidence comes from the observation that the BB-like cells contact almost all available S-cone pedicles in their dendritic fields (nine S-cone pedicles in the dendritic fields of five BB-like cells; only one S-cone pedicle without dendritic contact). These incomplete S-cone contacts of the BB-like cells probably result from the unavailability of S-cones in their dendritic fields[12] because the S-cone population is sparse in the dorsal side of the rabbit retina. Although we cannot be sure whether dendritic branches without S-cone contact connect to M-cones or simply end without any cone input (given that some dendrites have claw structures indicative of contact with cone pedicles), this incomplete S-cone contact has been shown in primate retinas. [12]

Therefore, it is likely that some blue cone bipolar cells have no complete S-cone contact in the blue cone sparse area of the rabbit retina. This is also consistent with findings that blue cone bipolar cells in primates vary in the number of presynaptic S-cones.[11,12] Furthermore, though the depths of axon terminals of the BB-like cells in the IPL showed some discrepancies from the BB cells (Table 3), this variation may result from the slight compression of the IPL during microinjection of the INL neurons. Given that there is no clear morphologic difference between BB and BB-like cells except their complete S-cone connectivity, we believe these five BB-like cells should be categorized by BB cell type. This would give a total of seven blue cone bipolar cells identified in the present study.

TABLE 3. Summary of Wide-Field Types of Bipolar Cells Examined

		Axon Terminal			Dendrite		
Cell Type	No. Cells Injected	Field Area (μm^2)	Diameter (μm)	Stratification (% IPL)	Field Area (μm^2)	Diameter (μm)	Cone Connectivity
BB	2	4,340 ± 1,520	73 ± 13	5–15	1,580 ± 520	44 ± 8	S
BB-like	5	3,270 ± 1,440	63 ± 14	10–40	1,710 ± 390	46 ± 5	S*
WFa	6	4,990 ± 780	79 ± 6	20–40	1,850 ± 260	48 ± 3	S, M
WFb	4	5,130 ± 1,000	80 ± 8	45–60	1,910 ± 470	49 ± 6	S, M

Axon terminal and dendritic field areas were computed from a polygon connecting the tips of the axon terminal and dendritic endings, respectively. Diameters of the axon terminals and dendritic fields were derived from the axonal field and the dendritic field area, accordingly. BB, blue cone bipolar cells; BB-like, BB-like cells; WFa, OFF-type wide-field bipolar cells; WFb, ON-type wide-field bipolar cells. Note that WFa and WFb were not the wa and wb identified in Famiglietti.[4]

* BB-like cells have incomplete S-cone contacts because of the unavailability of S-cones in the dorsal side of the rabbit retina.

Comparisons of Wide-Field Bipolar Cells

Two types of wide-field bipolar cells have been described previously in the rabbit retina, including the OFF-type[3,4] and the ON-type.[2,4,22] Among them, Famiglietti[4] indicated that the wa (OFF-type) and the wb (ON-type) bipolar cells are potential candidates involved in color information processing in the rabbit retina. In Famiglietti's description, "each wide-field bipolar cell gives rise to 1 to 4 relatively thick and extensive primary dendrites which rarely branch before giving off a small terminal cluster. . . . The dendrites of wa bipolar cells terminate at these clusters, but the dendrites of wb bipolar cells extend for considerable distance beyond the terminal cluster" (Ref. 4, p. 1561). Although Famiglietti4 did not give detailed images of these wa and wb cells, according to these morphologic properties, the BB and BB-like cells are similar to Famiglietti's wa bipolar cells, whereas the WFa and the WFb cells observed in the present study are clearly different from the wa and wb cells described in Famiglietti' [4] respectively. However, there is no ON-type wide-field bipolar cell whose morphology is similar to that of Famiglietti's wb bipolar cells (or equivalently the biocytin-labeled bipolar cells in Jeon and Masland[22] and the wide-field BP cells in MacNeil et al.[2]) found in the present study.

McGillem and Dacheux[3] reported an OFF-type of wide-field bipolar cells named CBwa1 in the rabbit retina that they believed corresponded to the DAPI-Ba3 bipolar cell described byMills and Massey.[30] The axon terminals of the CBwa1 bipolar cells extended 60 μm from the soma, suggesting they are in fact wide-field cells and may be the BB cells that we observed. We cannot conclude this with certainty because the cone contacts of CBwa1 were not identified, and their responsiveness to blue light was not studied. Furthermore, the CBwa1 bipolar cells are equivalent to the $CBa1_w$ cells in MacNeil et al.,[2] and this implies that the $CBa1_w$ cells could be homologous to the BB cells. We are not certain about this possibility either, though the $CBa1_w$ cells in MacNeil et al.[2] and in the present study all had

axon terminals that spanned less than 50 μm in diameter, which qualifies them as medium-field bipolar cells (Table 2). The WFa cells that we identified in the present study have dendritic and axonal features in common with the DAPIBa3 bipolar cells reported in Mills and Massey,[30] though the axon terminal stratifications are slightly different between the DAPI-Ba3 cells (20% of the IPL) and the WFa cells (20%–40% of the IPL). These apparent axonal size and stratification differences between WFa cells (Table 3) and DAPI-Ba3 cells could be attributed to retinal locations because our sampling was restricted in the dorsal retina. If the WFa cells are indeed homologous to the DAPI-Ba3 cells, the CBwa1 cells3 and the $CBa1_w$ cells[2] described may not be equivalent to the BB cells found in the present study.

Similarly, Jeon and Masland[22] found an ON-type of widefield bipolar cells in the rabbit retina by selective biocytin labeling. These cells were originally concluded to be the same cells as Famiglietti's wb bipolar cells. Most recently, MacNeil and Gaul (MacNeil MA et al. *IOVS* 2006;47:ARVO E-Abstract 147) showed that the biocytin-labeled cells indeed contact S-cones exclusively and are the ON-type blue cone bipolar cells. Interestingly, the WFb cells identified in the present study have no counterpart in previous studies. Given that the dendritic and axonal morphologies of the WFb cells and the DAPI-Ba3 cells are similar, it is likely that they are variants of the same basic structure (e.g., they might be ON and OFF versions of the same cell). Our sampling confined in the dorsal retina may contribute to this apparent difference.

Several distinct types of wide-field bipolar cells have been reported in other mammals. For example, Kolb et al.[33] found cb7 and cb8 wide-field bipolar cells in the cat retina, whose axonal arbors ramify in layers 2 and 5 of the IPL, respectively. The cb8 cells in cats probably have the same cell types as the wide-field bipolar cells identified in rabbits by Jeon and Masland[22] and the wb cells in Famiglietti,[4] but whether the cb7 cells in cats and the BB cells revealed in the present study are the same cell types is not certain. In the monkey retina, a giant bipolar cell has been described in which the axon arbors ramify in both sublamina a and sublamina b of the IPL, yielding a bistratified wide-field bipolar cell.[34] However, no bistratified bipolar cell has been found thus far in the rabbit retina.[2–4] In the ground squirrel, the B7 wide-field bipolar cells[35] were found to be homologous to the wa cells in rabbits,[4] and it has been indicated that they might be responsible for carrying blue cone signals to ganglion cells. Although wide-field bipolar cells are common in mammalian retinas, their synaptic connections and functions are poorly understood.

Color Circuitry in the Rabbit Retina

It has been shown that rabbits have spectral opponent neurons in the retinal level[19–21] and in the lateral geniculate nucleus level.[36] Behavioral evidence[15] and ERG experiments[17] also support that rabbits are capable of color discrimination. However, the color circuitry in the rabbit retina that is responsible for carrying spectral information to the brain has not been systematically examined. Based on the axon terminal stratification pattern in the IPL, the BB cells identified in the present study can be assumed to provide the blue-OFF signal to the underlying ganglion cells. Although Caldwell and Daw[19] only recorded the blue ON-center and green-OFF surround ganglion cells (they classified these color-coded ganglion cells as a subgroup of the ON-type X cells) and they never encountered the blue OFF-center and green-ON surround ganglion cells in the rabbit retina, De Monasterio[20] has identified the blue ON- and the blue OFF-types in the arterially perfused eyecup preparation using intracellular recordings. In addition, he also found some color-coded ganglion cells with mixed

ON-depolarizing inputs from blue cones and green cones and antagonistic OFF-depolarizing inputs from only blue cones or green cones. Furthermore, Vaney et al.[21] also reported the blue OFF-center and green ON-surround ganglion cells in the rabbit retina. Therefore, the blue-OFF signal presumably carried by the BB cells identified in the present study may represent the OFFdepolarizing input to the color-coded ganglion cells reported in De Monasterio[20] and Vaney et al.,[21] though one recent study showed that certain bipolar cells arborizing in sublamina 1 of the cat retina could provide inhibitory input to the targeted ganglion cells.[37] Despite these early physiological identifications of color-selective ganglion cells in the rabbit retina, the morphologic characteristics of these ganglion cells are not clear. Rockhill et al.[38] suggested that the small bistratified ganglion cells (the G3 cells) identified in the rabbit retina could potentially receive green/blue opponent signals from the ON/OFF sublaminas of the IPL, though the G3 cells only showed ON response with stimulation of a white spot.[39] Whether the bistratified G3 ganglion cells are color-coded ganglion cells in the rabbit retina remains to be shown.

Famiglietti[4] proposed that two types of wide-field bipolar cells are involved in rabbit color vision. If the OFF-type BB cells identified in the present study are indeed the same cell types as the wa bipolar cells described in Famiglietti,[4] one would expect other ON-type BB cells in the rabbit retina equivalent to the wb cells reported in Famiglietti[4] to be found. Although we injected seven BB and BB-like cells in the present study, we have not yet identified any ON-type BB cell. Despite missing the ON-type blue cone bipolar cell, we identified convincingly an OFF-type of wide-field bipolar cells responsible for conveying the short-wavelength signals from the outer retina to the inner retina in rabbits. Together with the recent finding that the biocytin-labeled wide-field bipolar cell is the ON-type blue cone bipolar cell (MacNeil MA et al. *IOVS* 2006;47:ARVO E-Abstract 147), this essentially confirms Famiglietti's early proposal, [4] in contrast to other blue cone bipolar cells identified in primates,[12] mice,[13] and ground squirrels.[14]

Blue Cone Bipolar Cells in Other Mammals

Although the morphologic identity of the ON-type blue cone bipolar cells in primates has been well studied,[6,9] the existence of its counterpart (the OFF-type blue cone bipolar cells) has been controversial.[40] Recent evidence indicates that a subpopulation of the midget bipolar cells could be the long-sought OFF-type blue cone bipolar cells in the monkey retina.[41,42] A related study also suggests that the melanopsin-expressing ganglion cells in primates could receive blue inputs from sublamina 1 of the IPL.[43] However, the morphologies of these two types of blue cone bipolar cells in primates are not the ON and OFF versions of the same cell. Furthermore, ON-type blue cone bipolar cells have been confirmed recently in the mouse and ground squirrel retinas in which the morphologic features are similar to the BB cells identified in the primate retina.[13,14] Given the evolutionarily conserved blue-yellow circuitry in mammalian retinas and the great similarity of the ON-type blue cone bipolar cells identified in monkeys and other mammals, it is natural to assume that all mammals have the same type of blue cone bipolar cells. The fact that rabbits have the OFF-type blue cone bipolar cell in addition to the ON-type blue cone bipolar cell identified recently (MacNeil MA et al. *IOVS* 2006; 47:ARVO E-Abstract 147) indicates that both ON and OFF blue cone bipolar cells are equally important for transmitting color signals in the rabbit retina.

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